CPAL
Central **P**ennsylvania **A**lliance **L**aboratory

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ENA Screens
- Now Performed at CPAL –

Contact: Stephanie Frey, 717.852.4768
Operations Manager, Clinical Pathology, CPAL

Dr. Jennifer Thebo, 717.851.3210
Director, Clinical Pathology, CPAL

Affected Tests:

Mnemonics:	**ROLA**	**ANTI LA**	**ANTI RNP**	**ANTI RO**	**ANTI SM**	**SMRNP**	**ENA ABS**
Test Name:	ENA: Anti-Ro/La	ENA: Anti-La (SS-B Sjogren's Antibody)	ENA: Anti-RNP (ribonucleo-protein)	ENA: Anti-Ro (SS-A Sjogren's Antibody)	ENA: Anti-Sm (Smith)	ENA: Anti-Sm/RNP	ENA: ENA Group (Anti-Sm, Anti-RNP, Anti-Ro, Anti-La)
Test Number:	**3000820**	**3000816**	**3000812**	**3000814**	**3000810**	**3000818**	**3000822**
Includes:	Anti-Ro Anti-La	Anti-La	Anti-RNP	Anti-Ro	Anti-Sm	Anti-Sm Anti-RNP	Anti-Sm Anti-RNP Anti-Ro Anti-La
Specimen:	1.0 mL Serum, Plastic SST. Stable at 2-8ºC for up to 2 days.						

Effective Date: Testing will begin on May 19, 2014 with samples received on May 15, 2014.

Performed: Monday, Wednesday, Friday

Reference Range: Negative

Method Change: ENA testing was previously conducted at CPAL using the NOVA Gel method. In 2011, CPAL began sending the testing to Quest due to scheduling constraints and the manual nature of the Gel method. With the availability of a more automated method, the testing can now be done at CPAL once again.

Background:
The determination of antinuclear antibodies (ANA) is of central importance for the clinical diagnosis of connective tissue diseases. Sm antibodies offer a highly specific, but comparatively insensitive, clinical marker for SLE. Indeed, their presence constitutes one of the revised ACR criteria for diagnosis, even though their overall prevalence ranges from 20% to 30% in SLE. U1-snRNP antibodies typically appear in both SLE and mixed connective tissue disease (MCTD, Sharp Syndrome). In MCTD, the presence of U1-snRNP antibodies is required for diagnosis, whereas they occur in only 30 to 40% of SLE patients. Although the anti-U1snRNP immune response comprises antibodies against all 3 protein components (70 kDa, A, C) 70 kDa antibodies – particularly in case of high titers – may be more specific for MCTD, as they have been reported to occur less frequently in SLE (approximately 12%) than antibodies against A or C proteins (approximately 23%). Several studies have shown that an anit-U1-snRNP response in the absence of 70 kDa antibodies is strongly associated with SLE.

Detection of SS-A/Ro antibodies is of interest and significance for the clinical diagnosis of SLE (prevalence 40-50%) and Sjogren's syndrome (prevalence 60-75% for primary Sjogren's syndrome). They have been reported to occur in tight association with certain disease subsets, such as subacute cutaneous LE, neonatal lupus erythematosus or vasculitis in Sjogren's syndrome. As anti-SS-A/Ro may be the only antibody present in many patients with SLE or Sjogren's syndrome, failure to measure anti-SS-A/Ro leaves a diagnostic void which cannot be filled by other tests.

SS-B/La antibodies are the serological hallmark of Sjogren's syndrome, but a small proportion of the patients remain anti-SS-B/La negative. Reported in 6-15% of sera from SLE patients, SS-B/La antibodies are associated with a lower prevalence of dsDNA antibodies and renal disease in these patients. Although a strong association of neonatal lupus erythematosus (NLE) with anti-SS-A/Ro was recognized first, the majority of mothers with babies with NLE are now known to have serum SS-B/La antibodies as well.

Method Principle:
ENA testing is performed on the Phadia ImmunoCap 250 system using EliA technology. The EliA wells are coated with human recombinant U1RNP (RNP 70, A, C), SS-A/Ro (60 kDa, 52 kDa), SS-B/La or native purified Sm proteins. If present in the patient's specimen, antibodies to the antigens bind to their specific antigen. After washing away non-bound antibodies, enzyme-labeled antibodies against human IgG antibodies (EliA IgG Conjugate) are added to form an antibody-conjugate complex. After incubation, non-bound conjugate is washed away and the bound complex is incubated with Development Solution. After stopping the reaction, the fluorescence in the reaction mixture is measured. The higher the response value, the more specific IgG is present in the specimen. To evaluate test results, the response for patient samples is compared directly to the response for calibrators.

Results Interpretation:
The Phadia reports ENA results in EliA U/mL. Results are reported as Negative, Equivocal, or Positive based upon the interpretive criteria presented in Table 1 below.

Table 1	**Negative**	**Equivocal**	**Positive**
Sm	< 5 EliA U/mL	5-10 EliA U/mL	>10 EliA U/mL
U1RNP	< 5 EliA U/mL	5-10 EliA U/mL	>10 EliA U/mL
Ro (SS-A)	< 7 EliA U/mL	7-10 EliA U/mL	>10 EliA U/mL
La (SS-B)	< 7 EliA U/mL	7-10 EliA U/mL	>10 EliA U/mL

Limitations:
A definitive clinical diagnosis should not be based on the results of a single diagnostic method, but should only be made by the physician after all clinical and laboratory findings have been evaluated.

Validation Data:
Precision
Manufacturer's criteria for precision are within run CV<10%, between run CV<12% for results within the equivocal and positive ranges. No claims are made for results in the negative range. Laboratory evaluation criteria for negative results were within run CV<15% and between run CV<20%. All precision criteria were met (Tables 2 and 3).

Table 2 Within Run Precision

Test	**EliA U/mL**	**%CV**	**EliA U/mL**	**%CV**	**EliA U/mL**	**%CV**
SM	25.2	3.3%	35.4	4.3%	0.1	0%
U1RNP	63.4	3.6%	82.0	3.4%	0.8	10.5%
Ro (SS-A)	232.0	1.9%	52.8	2.5%	<0.3	0%
La (SS-B)	167.8	4.1%	24.6	4.6%	<0.3	0%

Table 3 Between Run Precision

Test	**EliA U/mL**	**%CV**	**EliA U/mL**	**%CV**	**EliA U/mL**	**%CV**
SM	26.2	5.0%	35.9	3.6%	0.1	0%
U1RNP	62.2	5.2%	81.5	4.0%	0.9	16.4%
Ro (SS-A)	221.8	6.0%	51.0	4.2%	0.3	0.1%
La (SS-B)	164.7	6.8%	23.7	5.6%	<0.3	0%

Method Comparison

A total of 53 specimens were split and processed utilizing Phadia 250 EliA ENA asays. The results were compared to those from Quest Diagnostics (Table 4).

Table 4: Phadia vs Quest ENA

		Quest		
	SM	POS	neg	Total
Phadia	POS	3	0	3
	neg	5	19	24
	Total	8	19	27

sensitivity 37.5%
specificity 100.0%

		Quest		
	RNP	POS	neg	Total
Phadia	POS	10	0	10
	neg	5	14	19
	Total	15	14	29

sensitivity 66.7%
specificity 100.0%

		Quest		
	Ro	POS	neg	Total
Phadia	POS	15	0	15
	neg	0	16	16
	Total	15	16	31

sensitivity 100.0%
specificity 100.0%

		Quest		
	La	POS	neg	Total
Phadia	POS	9	0	9
	neg	7	16	23
	Total	16	16	32

sensitivity 56.3%
specificity 100.0%

There were a total of 17 discordant results among the SM, RNP, and La assays. The Ro assay had 100% agreement with the Quest results. To resolve the discrepancies a double diffusion test system, NOVA Gel, was obtained from INOVA. NOVA Gel was the method used at CPAL before the testing was sent to Quest Diagnostics. The gel plates were set and read by a technologist who performed the testing when it was in house. Eleven of the discrepancies were resolved using the NOVA Gel kit. The remaining six specimens were QNS and could not be tested using NOVA Gel. There was 100% agreement with NOVA Gel versus Phadia 250 for the discordant results. Results following resolution are shown in Table 5.

Table 5: After Resolution with NOVA gel method

		Quest + NOVA Gel		
	SM	POS	neg	Total
Phadia	POS	6	0	6
	neg	0	19	19
	Total	6	19	25

sensitivity 100.0%
specificity 100.0%

		Quest + NOVA Gel		
	RNP	POS	neg	Total
Phadia	POS	13	0	13
	neg	0	14	14
	Total	13	14	27

sensitivity 100.0%
specificity 100.0%

		Quest + NOVA Gel		
	Ro	POS	neg	Total
Phadia	POS	15	0	15
	neg	0	16	16
	Total	15	16	31

sensitivity 100.0%
specificity 100.0%

		Quest + NOVA Gel		
	La	POS	neg	Total
Phadia	POS	14	0	14
	neg	0	16	16
	Total	14	16	29

Sensitivity 100.0%
specificity 100.0%

REFERENCES:

EliA U1RNP package insert, 250-5501-03/US, Phadia. August 2010.

EliA Sm package insert, 250-5502-03/US, Phadia. August 2010.

EliA Ro package insert, 250-5503-03/US, Phadia. August 2010.

EliA La package insert, 250-5504-05/US, Phadia. November 2011.